EFSA Panel on food contact materials, enzymes, flavourings and processing aids (CEF); Scientific Opinion on Flavouring Group Evaluation 74, Revision 1 (FGE.74Rev1): Consideration of Simple Aliphatic Sulphides and Thiols evaluated by the JECFA (53rd and 61st meeting) Structurally related to Aliphatic and Alicyclic Mono-, Di-, Tri-, and Polysulphides with or without Additional Oxygenated Functional Groups from Chemical Group 20 evaluated by EFSA in FGE.08Rev1 (2009)

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 74, Revision 1 (FGE.74Rev1):

Consideration of Simple Aliphatic Sulphides and Thiols evaluated by the JECFA (53rd and 61st meeting) Structurally related to Aliphatic and Alicyclic Mono-, Di-, Tri-, and Polysulphides with or without Additional Oxygenated Functional Groups from Chemical Group 20 evaluated by EFSA in FGE.08Rev1 (2009)[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The JECFA has evaluated a group of 12 simple aliphatic sulphides and thiols at the 61st meeting and seven trisulphides in a group of simple aliphatic and aromatic sulphides and thiols at the 53rd meeting. One of the substances evaluated by the JECFA at its 61st meeting is not in the Register (spiro[2,4-

1 On request from the Commission, Question No EFSA-Q-2009-00954, adopted on 30 September 2010.

2 Panel members: Arturo Anadon, David Bell, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle.

3 Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion:Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Jørn Gry, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific Opinion.

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dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3'-(1'-oxa-2'-methyl)-cyclopentane], JECFA-no: 1296). Accordingly this consideration will deal with 18 JECFA evaluated substances.

The Panel concluded that the 18 substances in the JECFA flavouring group of simple aliphatic sulphides and thiols are structurally related to the group of 66 aliphatic and alicyclic mono-, di-, and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in the Flavouring Group Evaluation 08, Revision 1(FGE.08Rev1).

The Panel agrees with the outcome of the application of the Procedure performed by the JECFA for eight of the 18 aliphatic sulphides and thiols [FL-no: 12.179, 12.198, 12.212, 12.238, 12.239, 12.255, 12.257 and 12.291].

For two tertiary thiols, 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] and 2-mercapto-2-methylpentan-1-ol [FL-no: 12.241], the Panel concluded that they should not be evaluated through the Procedure, as they are structurally related to three tertiary thiols evaluated in FGE.08Rev1 which could not be evaluated through the Procedure due to concern with respect to genotoxicity *in vitro.*

For the eight tri- and polysulphides [FL-no: FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280] the Panel did not agree with the JECFA that appropiate studies were available for deriving NOAELs, and accordingly additional data are required for these eight substances.

For two substances [FL-no: 12.045 and 12.155] the JECFA evaluation is only based on MSDI values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For one substance use levels have been provided by the Industry. For the remaining 17 substances use levels must be provided. These are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 18 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for 10 of the 18 JECFA evaluated substances. For seven substances [FL-no: 12.009, 12.020, 12.045, 12.169, 12.238, 12.239 and 12.291] information on secondary components and/or composition of mixture is requested. For six substances [FL-no: 12.009, 12.020, 12.023, 12.045, 12.074 and 12.155] no solubility in ethanol and/or solubility in water is available. Finally, the European production volumes are not available for [FL-no: 12.045 and 12.155].

Thus, for 10 substances [FL-no: 12.009, 12.020, 12.023, 12.045, 12.074, 12.155, 12.169, 12.238, 12.239 and 12.291] the Panel has reservations (no European production volumes are available, preventing them to be evaluated using the Procedure, and/or information on specifications). For two substances [FL-no: 12.169 and 12.241] the Procedure should not be applied until adequate genotoxicity data become available and for eight substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280] additional toxicity data are required.

For the remaining five of the 18 JECFA evaluated simple aliphatic sulphides and thiols [FL-no: 12.179, 12.198, 12.212, 12.255 and 12.257] the Panel agrees with JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

KEY WORDS

Safety, flavourings, aliphatic, sulphides, thiols, JECFA, 53rd meeting, 61st meeting

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th 68th and 69th meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register which was adopted by Commission Decision 1999/217 EC (EC, 1999a) and its consecutive amendments.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999a), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available,

meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?")" (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999a), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

At its 61st meeting the JECFA evaluated a group of 12 flavouring substances consisting of simple aliphatic sulphides and thiols. One substance was not in the Register. The remaining 11 flavouring substances have originally been considered by EFSA in the FGE.74 (EFSA, 2009t).

FGE	Opinion Adopted by EFSA	Link	No. of Candidate Substances
FGE.74	January 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902376194.htm	11
FGE.74Rev1	September 2010		18

In the present revision of FGE.74, FGE.74Rev1, there has been a reassessment of four candidate substances due to sub-grouping of the substances based on the type of sulphur-containing functional groups. This is in accordance with what has been done in FGE.08Rev1 and in FGE.91, which also consider substances with sulphur-containing functional groups. The candidate substances in FGE.74Rev1 that have been reassessed due to this are [FL-no: 12.179, 12.198, 12.212 and 12.280]. The outcome of the evaluation is explained in Section 4.3.

Furthermore, the present revision includes the assessment of seven additional substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155] evaluated by the JECFA at the 53rd meeting in 1999. The reason for the inclusion of these seven substances is explained in Section 1.1.2.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 12 flavouring substances consisting of simple aliphatic sulphides and thiols at the 61st meeting.

The JECFA has at the 53rd meeting (JECFA, 2000c), before 2000, evaluated a group of 137 flavouring substances consisting of simple aliphatic and aromatic sulphides and thiols with and without an additional oxygenated functional group. Seven of these 137 substances are tri- or polysulphides [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155], which will be considered in the present FGE.

1.1.2. EFSA Considerations

Of the in total 19 substances mentioned above, one substance evaluated by the JECFA at its 61st meeting is not in the Register (spiro[2,4-dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3'-(1'-oxa-2'-methyl)-cyclopentane], JECFA-no: 1296). This consideration will therefore only deal with 18 JECFA evaluated substances. Eleven substances from the 61st meeting, 2003, and seven tri- and polysulphides from the 53rd meeting, 1999.

The Panel concluded that the substances in the JECFA flavouring group of simple aliphatic sulphides and thiols are structurally related to the group of aliphatic and alicyclic mono-, di-, tri- and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in the Flavouring Group Evaluation 08, Revision 1 (FGE.08Rev1). Depending on the type of sulphur-containing functional groups, the substances in FGE.08Rev1 were subdivided into ten subgroups:

I *Acyclic sulphides*

II *Cyclic sulphides*

III *Monothiols, including tertiary monothiols*

IV *Dithiols*

V *Acyclic and cyclic disulphides*

VI *Acyclic tri- and polysulphides*

VII *Mono-, di-, tri- and polysulphides with thioacetal structure*

VIII *Thioesters*

IX *Thioic acid*

X *Sulphoxides/sulphones and sulphonates.*

The 18 JECFA evaluated substances in the present FGE will be considered in concordance with these EFSA defined subgroups.

Comment on Subgroup VI (Acyclic tri- and polysulphides)

During the evaluation of the candidate substances in FGE.08Rev1, it was recognised that tri-and polysulphides (subgroup VI) may form reactive metabolites through reaction with endogenous thiols forming a thiol and a hydropersulphide or perthiol. Compared to thiols, perthiols may be strong reducing agents, forming reactive products when exposed to oxidants. Based on the above information it was concluded that tri- and polysulphides could not be covered by No Observed Adverse Effect Levels (NOAELs) for disulphides, due to the formation of more reactive metabolites.

The Panel noted that in FGE.08Rev1 seven supporting substances are tri- or polysulphides [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155]. These substances were evaluated by JECFA before the year 2000[4] (accepted at step B4 based on NOAELs derived from studies with disulphides), and therefore not included in the consideration performed by EFSA on the JECFA evaluated substances in FGE.74.

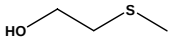
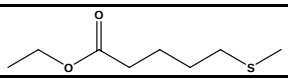
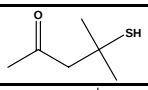
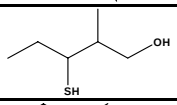
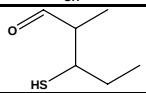
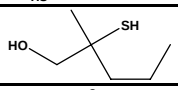
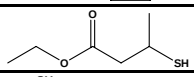
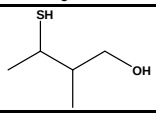
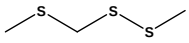
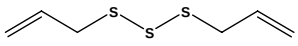
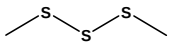
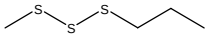
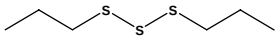
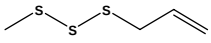
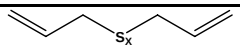
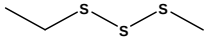
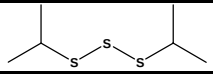
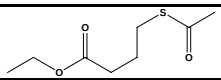
The decision taken in FGE.08Rev1 has accordingly impact on the tri- and polysulphides in FGE.74 (one substance [FL-no: 12.280]) as well as those evaluated by the JECFA at its 53rd meeting, before 2000 (seven substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155]), which are therefore included in this revision of FGE.74.

Distribution of the FGE.74Rev1 substances into subgroups

[4] For flavouring substances evaluated by the JECFA before 2000 it is laid down in Commission Regulation (EC) 1565/2000 (EC, 2000a) that if they are considered acceptable at the current estimated intake by the JECFA and comply with the general use criteria, they could be included in the list of authorised substances without undergoing a separate evaluation for the time being.

The 18 JECFA evaluated substances in this FGE have been assigned to five subgroups, in accordance with the subdivision in FGE.08Rev1. This subdivision is shown in Table 1.1 below.

Table 1.1: Allocation of the 18 JECFA evaluated substances into subgroups according to subdivision in FGE.08Rev1

FL-no:	Register name	Structural formula
I	***Acyclic sulphides***	
12.179	2-(Methylthio)ethan-1-ol	
12.212	Ethyl-5-(methylthio)valerate	
III	***Monothiols***	
12.169	2-Methyl-4-oxopentane-2-thiol	
12.238	3-Mercapto-2-methylpentan-1-ol	
12.239	3-Mercapto-2-methylpentanal	
12.241	2-Mercapto-2-methylpentan-1-ol	
12.255	Ethyl 3-mercaptobutyrate	
12.291	3-Mercapto-2-methyl-1-butanol	
V	***Acyclic and cyclic disulphides***	
12.198	2,3,5-Trithiahexane	
VI	***Acyclic tri- and polysulphides***	
12.009	Diallyl trisulfide	
12.013	Dimethyl trisulfide	
12.020	Methyl propyl trisulfide	
12.023	Dipropyl trisulfide	
12.045	Methyl allyl trisulfide	
12.074	Diallyl polysulfides	 X=2,3,4 or 5
12.155	Methyl ethyl trisulfide	
12.280	Diisopropyl trisulphide	
VIII	***Thioesters***	
12.257	Ethyl 4-(acetylthio)butyrate	

1.2. Isomers

1.2.1. JECFA Status

Two substances have one chiral centre [FL-no: 12.241 and 12.255] and three substances have two chiral centres [FL-no: 12.238, 12.239 and 12.291] in the group of the JECFA evaluated sulphides and thiols.

1.2.2. EFSA Considerations

For the two stereoisomeric substances [FL-no: 12.241 and 12.255] the CAS register number (CASrn) specifies the stereoisomeric composition as racemates.

For the three substances with two chiral centres [FL-no: 12.238, 12.239 and 12.291] the composition of mixture of the stereoisomers has not been specified.

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all 18 substances (JECFA, 1999c; JECFA, 2003b). See Table 1.

1.3.2. EFSA Considerations

The available specifications are considered adequate for 8 of the 18 JECFA evaluated substances. For the six trisulphides [FL-no: 12.009, 12.020, 12.023, 12.045, 12.074 and 12.155] no solubility in ethanol is available and for two substances [FL-no: 12.020 and 12.045] no solubility in water. For four substances [FL-no: 12.009, 12.020, 12.045 and 12.169] the assay minimum is less than 95 % and further information on the composition is requested. For the three substances [FL-no: 12.238, 12.239 and 12.291] with two chiral centres the composition of the mixture of the stereoisomers has to be specified (see Section 1.2).

2. Intake Estimations

2.1. JECFA Status

For 16 of the 18 substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 3.1. For the remaining two substances production figures are only available for the USA.

2.2. EFSA Considerations

As production figures are only available for the USA for two substances, MSDI values for the EU cannot be calculated for these [FL-no: 12.045 and 12.155].

For one of the 18 JECFA evaluated substances [FL-no: 12.291] normal and maximum use levels have been provided by the Flavour Industry in accordance with the Commission Regulation (EC) No 1565/2000 (Flavour Industry, 2008b; EC, 2000a) (see Table 2.2.1). Based on the normal use levels,

the mTAMDI figure can be calculated (see Table 2.2.2). For calculation of mTAMDI figures, see e.g. FGE.03, Annex II (EFSA, 2004d).

Table 2.2.1 Normal and Maximum use levels (mg/kg) available for JECFA evaluated substances in FGE.74Rev1

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
12.291	-	0,1	-	0,01	-	-	-	0,1	0,1	-	-	-	0,1	-	-	-	0,1	0,1
	-	0,5	-	0,1	-	-	-	1	2	-	-	-	1	-	-	-	1	0,5

Table 2.2.2 Estimated intakes based on the MSDI- and the mTAMDI approach

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
12.013	Dimethyl trisulfide	1.1	0.02		Class I	1800
12.020	Methyl propyl trisulfide	0.21	0.1		Class I	1800
12.023	Dipropyl trisulfide	7.3	1		Class I	1800
12.155	Methyl ethyl trisulfide	ND	1		Class I	1800
12.169	2-Methyl-4-oxopentane-2-thiol	0.0085	0.02		Class I	1800
12.179	2-(Methylthio)ethan-1-ol	0.85	0.9		Class I	1800
12.198	2,3,5-Trithiahexane	0.026	0.04		Class I	1800
12.212	Ethyl-5-(methylthio)valerate	1.7	2		Class I	1800
12.238	3-Mercapto-2-methylpentan-1-ol	0.85	0.7		Class I	1800
12.239	3-Mercapto-2-methylpentanal	2.6	4		Class I	1800
12.241	2-Mercapto-2-methylpentan-1-ol	2.6	4		Class I	1800
12.255	Ethyl 3-mercaptobutyrate	3.4	4		Class I	1800
12.257	Ethyl 4-(acetylthio) butyrate	3.4	4		Class I	1800
12.280	Diisopropyl trisulphide	0.24	0.007		Class I	1800
12.291	3-Mercapto-2-methyl-1-butanol	0.061	2	17	Class I	1800
12.009	Diallyl trisulfide	3.5	0.02		Class II	540
12.045	Methyl allyl trisulfide	ND	0.9		Class II	540
12.074	Diallyl polysulfides	1.2	0.02		Class II	540

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken[5] from the JECFA (JECFA, 2000c; JECFA, 2004b)

Groups of male ICR mice were given two doses 48 hours apart of a mixture containing allyl sulfide [FL-no: 12.088], allyl disulfide (JECFA-no: 572), or diallyl trisulfide [FL-no: 12.009] in corn oil at doses of 10 or 20 mg/ml by gavage. The doses were estimated to provide 0.33 or 0.67 mmol/kg bw or 50 or 100 mg/kg bw on the basis of the composition of the mixture. No increase in the frequency of micronucleated polychromatic erythrocytes was seen in bone-marrow cells (Marks et al., 1992).

Erythro- and threo-3-mercapto-2-methylbutanol [FL-no: 12.291 (3-mercapto-2-methyl-1-butanol)] (50–5000 µg/plate) was evaluated for mutagenic activity in the modified Ames test with preincubation in the presence and absence of metabolic activation in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102 and TA1535. No genotoxic effects were observed (Gocke, 1997a).

For a summary of *in vitro / in vivo* genotoxicity data considered by the JECFA, see Table 2.1.

[5] The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed

3.2. Genotoxicity Studies - Text from FGE.08Rev1 (EFSA, 2009z)

In vitro / in vivo

Genotoxicity *in vitro* data are available for five of the 66 candidate substances: di-(1-propenyl)-sulphide (mixture) [FL-no: 12.298] (subgroup I), tetrahydrothiophene [FL-no: 15.102] (subgroup II); 2-methylpropane-2-thiol [FL-no: 12.174] (subgroup III); dibutyl disulphide [FL-no: 12.111] (subgroup V) and methyl methanethiosulphonate [FL-no: 12.159] (subgroup X). In addition studies are available on 14 supporting substances from subgroups I (1), II(1), III (4), IV (1), V (4), VIII (2) and X (1).

In vivo data are available for one candidate substance [FL-no: 12.159] (subgroup X) and for four supporting substances from subgroups I (1), III (1), V (I) and VI (1).

Only text from subgroups which are represented in the present FGE.74Rev1 is cited in the following:

Subgroup I (Acyclic sulphides)

In vitro data are available for the candidate substance, di-(1-propenyl)-sulfide [FL-no: 12.298]; Ames test: *S. typhimurium* TA98, TA100, TA102, TA1535, TA1537, 1-100 microgram/plate. Results were negative both with and without metabolic activation (Stien, 2005c).

Data are available only on the supporting substance diallyl sulphide [FL-no: 12.088]. Diallyl sulphide was negative in a limited bacterial reversion assay using one tester strain only (TA100) and provided equivocal results in an *in vitro* cytogenetic test in which increased incidences of cells with chromosomal aberrations and sister chromatid exchanges (SCEs), statistically significant but not dose related, were observed. *In vivo*, diallyl sulphide was evaluated as negative in a micronucleus test in mouse bone marrow, which was, however, not designed to evaluate the genotoxicity of the substance itself as it was tested in a mixture. Overall the data available do not allow evaluation of the genotoxicity of the substances in this subgroup.

Subgroup III (Monothiols)

2-Methylpropane-2-thiol [FL-no: 12.174] is reported to be negative in an Ames test. It is reported to be positive in a mouse lymphoma assay without metabolic activation and negative in the test with metabolic activation, and it is reported to be negative in an *in vitro* SCE assay. However, these studies are reported only as summaries (Phillips Petroleum Company, 1990a). Some details are available for methods but not for the results. Although the validity of these studies cannot be fully evaluated, the positive result in the mouse lymphoma assay raises concern with respect to the potential for genotoxicity of this tertiary thiol and structurally related compounds, i.e. 2-methylbutane-2-thiol [FL-no: 12.172].

The *in vitro* data available for the other substances in this subgroup do not provide indication of concern for genotoxicity.

Subgroup V (Acyclic and Cyclic di-sulphides)

Dibutyl disulphide [FL-no: 12.111] is reported to be negative in a mouse lymphoma assay (Dooley et al., 1987). However, the study is reported only as abstract, and thus, the validity cannot be evaluated.

Further data are available for the supporting substances diallyl disulphide [FL-no: 12.008], dimethyldisulphide [FL-no: 12.026], phenyl disulphide [FL-no: 12.043] and benzyl disulphide [FL-no: 12.081]. All substances were negative in the Ames test. In addition, diallyl disulphide was reported to be positive in a chromosomal aberration assay *in vitro*, with and without metabolic activation, and weakly positive in a SCE assay. However, the validity of these findings is doubtful as chromosomal

aberrations were only increased in conditions associated with extensive (> 90 %) lethality and because of the limitations of SCE in genotoxic hazard identification.

Subgroup VI (Acyclic tri- and polysulphides)

No genotoxicity information is available.

Subgroup VIII (Thioesters)

The *in vitro* data available on supporting substances provide no indication of concern for genotoxicity.

Conclusion on genotoxicity

Most *in vitro* and *in vivo* studies are of limited or insufficient quality and provide only limited information.

The available data raise concern with respect to genotoxicity of two tertiary thiols [FL-no: 12.172 and 12.174], included as candidate substances in subgroup III. Hydrolysis of the candidate substance 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057], included in subgroup VII, leads to the formation of a tertiary thiol structurally related to the above-mentioned compounds. Therefore, there is also concern with respect to genotoxicity of this candidate substance. The Panel noted that in FGE.08 five of the supporting substances were tertiary thiols [FL-no: 12.038, 12.085, 12.137, 12.138 and 12.145] for which a concern for genotoxicity has been raised in FGE.08Rev1. These supporting substances have been evaluated by JECFA at the 53rd meeting (JECFA, 2000b; JECFA, 2000c) and are not scheduled for evaluation by EFSA. However, these substances should be considered by Panel based on the outcome of the evaluation of the two candidate tertiary thiols [FL-no: 12.172 and 12.174] in FGE.08Rev1. In addition, genotoxicity of the candidate substance methyl methanethiosulfonate [FL-no: 12.159], included in subgroup X, could not be assessed from the data available. However, due to the similarity with methylmethane sulphonate, a direct acting mutagen and carcinogen, there is concern with respect to genotoxic potential of this candidate substance.

Therefore, the Panel decided that the Procedure could not be applied to the four candidate substances [FL-no: 12.159, 12.172, 12.174 and 16.057] until adequate *in vivo* genotoxicity data become available that may clear the concern for genotoxicity.

The other *in vitro / in vivo* genotoxicity data available, often from limited or poorly reported studies, do not provide clear indication of concern for genotoxicity for the remaining candidate substances included in the present evaluation.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA, see Tables 2.2 and 2.3.

3.3. EFSA Considerations

In FGE.08Rev1 concern was raised with respect to genotoxicity for two tertiary thiols [FL-no: 12.172 and 12.174] and one substance that is hydrolysed to a tertiary thiol [FL-no: 16.057] and accordingly these substances were not evaluated using the Procedure. The two JECFA evaluated tertiary thiols [FL-no: 12.169 and 12.241] in FGE.74Rev1 are also considered to be structurally related to the tertiary thiols in FGE.08Rev1 and thus cannot be evaluated using the Procedure either. Therefore additional data are required. For the remaining 16 of the 18 substances in FGE.74Rev1 the Panel considers that the genotoxicity data available do not preclude evaluating these substances through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 18 Simple Aliphatic Sulphides and Thiols evaluated by the JECFA (JECFA, 2000c; JECFA, 2004b):

According to JECFA 15 of the 18 substances belong to structural class I and three to structural class II using the decision tree approach presented (Cramer et al., 1978).

None of the substances could be anticipated to be metabolised to innocuous products and were evaluated via the B-side of the Procedure. The estimated daily per capita intakes of the 18 flavouring substances are below the threshold of concern for structural class I and II, and a No Observed Adverse Effect Level (NOAEL) exists to provide an adequate margin of safety to the estimated intake as flavouring substances (step B4).

Step B4. For erythro- and threo-3-mercapto-2-methylbutanol [FL-no: 12.291], the NOEL of 0.7 mg/kg body weight per day for the structurally related substance 2-mercapto-3-butanol [FL-no: 12.024] from a 92-day study in rats fed by gavage (Cox et al., 1974a) provides an adequate margin of safety (>10.000) in relation to known levels of intake of this agent. This NOEL is also appropriate for the structurally related agents (±)-2-mercapto-2-methylpentan-1-ol [FL-no: 12.241], 3-mercapto-2-methylpentan-1-ol (racemic) [FL-no: 12.238], 3-mercapto-2-methylpentanal [FL-no: 12.239], and (±)-ethyl 3-mercaptobutyrate [FL-no: 12.255], because they are all acyclic thiols with oxidized side-chains that are anticipated to undergo oxidation or hydrolysis and subsequent metabolism via similar metabolic pathways.

For 4-mercapto-4-methyl-2-pentanone [FL-no: 12.169 (2-methyl-4-oxopentane-2-thiol)], the NOEL of 1.9 mg/kg bw per day for the structurally related substance 3-mercapto-2-pentanone [FL-no: 12.031] administered to rats by gavage in a 92-day study (Morgareidge, 1971b) provides an adequate margin of safety (> 10,000) in relation to known levels of intake of this agent.

For ethyl 4-(acetylthio)butyrate [FL-no: 12.257], the NOEL of 6.5 mg/kg bw per day reported in a 13-week study in rats (Shellenberger, 1970b) fed with the structurally related substance ethylthioacetate [FL-no: 12.018] provides an adequate margin of safety (> 10,000) in relation to known levels of intake of this agent.

For 2-(methylthio)ethanol [FL-no: 12.179], the NOEL of 1.4 mg/kg bw per day reported in a 13-week study in rats (Cox et al., 1979) fed by gavage with the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal [FL-no: 12.087] provides an adequate margin of safety (>10,000) in relation to known levels of intake of this agent. This NOEL is also appropriate for the structurally related agent ethyl-5-(methylthio)valerate [FL-no: 12.212], which is also an acyclic sulphide with an oxidized side-chain that is anticipated to undergo oxidation and subsequent metabolism via similar pathways.

For 2,3,5-trithiahexane [FL-no: 12.198], the NOEL of 0.3 mg/kg bw per day reported in a 13-week study (Mondino, 1981a) in rats fed with the structurally related substance 3-methyl-1,2,4-trithiane [FL-no: 15.036] provides an adequate margin of safety (> 10,000) in relation to known levels of intake of this agent.

For diisopropyl trisulphide [FL-no: 12.280], the NOEL of 4.8 mg/kg bw per day reported in a 13-week study (Morgareidge & Oser, 1970c) in rats fed by gavage with the structurally related substance dipropyl trisulphide [FL-no: 12.023] provides an adequate margin of safety (>100,000) in relation to known levels of intake of this agent.

For diallyl trisulfide [FL-no: 12.009] and dipropyl trisulfide [FL-no: 12.023], the NOELs of 4.6 mg/kg bw per day and 4.8 mg/kg bw per day, respectively were reported in a 90 days study (Morgareidge & Oser, 1970c; Morgareidge & Oser, 1970d) at a single dose, which gave adequate margins of safety for

[FL-no: 12.013, 12.020, 12.045, 12.074 and 12.155]. The dose that had no effect is more than 10.000 times greater than the estimated per capita intake in Europe and more than 100.000 times higher than the estimated per capita intake in the United States.

In conclusion the JECFA evaluated all substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 18 simple aliphatic sulphides and thiols are summarised in Table 3.1.

4.2. Application of the Procedure to 66 Aliphatic and Alicyclic Mono-, Di-, Tri-, and Polysulphides with or without Additional Oxygenated Functional Groups by EFSA EFSA in FGE.08Rev1 (EFSA, 2009z):

For two of the candidate substances, 2-methylpropane-2-thiol [FL-no: 12.174] (subgroup III) and methyl methanethiosulphonate [FL-no: 12.159] (the only substance in subgroup X), there is indication of a genotoxic potential *in vitro*. Therefore, in the absence of further genotoxicity data, the Panel concluded that the Procedure could not be applied to these two substances, nor to the two structurally related candidates, 2-methylbutane-2-thiol [FL-no: 12.172] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057] (subgroup VII).

For four candidate substances, 3-mercaptooctanal [FL-no: 12.268] (subgroup III), 3-mercaptodecanal [FL-no: 12.269] (subgroup III), methanedithiol diacetate [FL-no: 12.271] (subgroup VIII) and 3,5-dimethyl-1,2-dithiolane-4-one [FL-no: 12.295] (subgroup V) no data on use as flavouring substances in Europe are available. Therefore no intakes in Europe can be estimated and accordingly the Panel concluded that the Procedure could not be applied to these four substances.

For the safety evaluation of the remaining 58 candidate substances from chemical groups 20 and 30 the Procedure as outlined in Annex I was applied based on the MSDI approach. The stepwise evaluations of the 58 substances evaluated through the Procedure are summarised in Table 3.2.

Step 1.

The candidate substances were classified following the procedure established by (Cramer et al., 1978). For the remaining 58 candidate substances, there are 38 substances classified into structural class I. Further 17 substances were classified into structural class II. The final three substances were classified into structural class III.

Step 2.

Step 2 requires consideration of whether metabolic pathways exist to metabolise the candidate substances to innocuous products at the expected levels of intake. The candidate substances may be biotransformed to reactive metabolites, such as thiols, sulphoxides and sulphones and, in consequence, they are not predicted to be metabolised to innocuous products. Therefore, the evaluation of all 58 candidate substances proceeds via the B-side of the evaluation Procedure (described in Annex I of FGE.08Rev1).

Step B3.

The 38 substances in structural class I have estimated European daily *per capita* intakes ranging from 0.0012 to 6.1 microgram, which is below the threshold of concern of 1800 microgram/person/day. The 17 substances evaluated through the Procedure in structural class II have estimated European daily *per capita* intakes ranging from 0.0024 to 2.4 microgram, which is below the threshold of concern for class II of 540 microgram/person/day. The three substances in structural class III have estimated European daily *per capita* intakes ranging from 0.012 to 3.7 microgram, which is below the threshold

of concern for class III of 90 microgram/person/day. Accordingly, all 58 candidate substances proceed to step B4.

Step B4.

No adequate studies on any candidate substances are available. Repeated-dose toxicity studies are available on some supporting substances, which, with very few exceptions, have been carried out testing only one dose, giving rise to no observed adverse effects. The results of adequate studies on supporting substances show a relatively high degree of variability in the reported NOAELs, ranging from 0.06 to 250 mg/kg bw/day.

The 18 candidate substances in subgroup I can be represented by the supporting substance dimethyl sulphide [FL-no: 12.006], for which an adequate 90-day subchronic study is available, indicating that no adverse effects were produced by the highest oral dose tested (250 mg/kg body weight (bw)/day), which can be considered as a NOAEL. The combined estimated daily *per capita* intake of 10 microgram for the 18 candidate substances in subgroup I corresponds to 0.17 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.5×10^6 can be calculated. The 18 candidate substances in subgroup I are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup II, no adequate toxicity study from which a NOAEL could be established was available, neither on the candidate substances nor on supporting substances. Therefore, the Panel concluded that additional data are required for the three cyclic sulphides in subgroup II [FL-no: 12.120, 15.102 and 15.125].

Within subgroup III, adequate 90-day subchronic studies are available for four supporting substances, 2-mercapto-3-butanol [FL-no: 12.024], cyclopentanethiol [FL-no: 12.029], 2,3- and 10-mercaptopinane [FL-no:12.035], and 2,6-(dimethyl)thiophenol [FL-no: 12.082], which can be considered representative of the seven remaining candidate substances in this subgroup to be evaluated through the Procedure. In the four studies, no adverse effects were produced by the highest oral dose tested ranging from 0.06 up to 0.7 mg/kg bw/day. By adopting a conservative approach the lowest value (0.06 mg/kg bw/day) can be considered as a NOAEL. The combined estimated daily *per capita* intake of 0.9 microgram for the seven candidate substances in subgroup III corresponds to 0.015 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 4×10^3 can be calculated. The seven candidate substances, evaluated through the Procedure, in subgroup III are accordingly not expected to be of safety concern at the estimated levels of intake.

The candidate substance in subgroup IV can be represented by two supporting substances, butane-2,3-dithiol [FL-no: 12.022], and octane-1,2-dithiol [FL-no: 12.034], for which adequate 90-day subchronic studies are available. In the two studies, no adverse effects were produced by the almost identical highest oral doses tested, that is 0.7 mg/kg bw/day, which can be considered as a NOAEL. The estimated daily *per capita* intake of 0.3 microgram for the one candidate substance in subgroup IV corresponds to 0.005 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.4×10^5 can be calculated. The candidate substance in subgroup IV is accordingly not expected to be of safety concern at the estimated level of intake.

Within subgroup V, adequate 90-day subchronic studies are available for two supporting substances dicyclohexyl disulphide [FL-no: 12.028] and benzyl methyl disulphide [FL-no: 12.068], which can be considered representative of the three candidate substances in this subgroup evaluated through the Procedure. In the two studies, no adverse effects were produced by the highest oral dose tested: 0.23 and 1.15 mg/kg bw/day. By adopting a conservative approach, the lowest value (0.23 mg/kg bw/day) can be considered as a NOAEL. The combined estimated daily *per capita* intake of 0.54 microgram for the three candidate substances in subgroup V corresponds to 0.009 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 2.6×10^4 can be calculated. The three candidate

substances in subgroup V are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup VI, no adequate toxicity study from which a NOAEL could be established was available, neither on the candidate substances nor on supporting substances. Therefore, the Panel concluded that additional data are required for the eight tri-, tetra- and polysulphides in subgroup VI [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167].

Within subgroup VII, adequate 90-day subchronic studies are available for two supporting substances, 3,5-dimethyl-1,2,4-trithiolane [FL-no: 15.025] and 2-methyl-4-propyl-1,3-oxathiane [FL-no: 16.030], which can be considered representative of the remaining nine candidate substances, evaluated through the Procedure, in this subgroup to be evaluated through the Procedure. In the two studies, no adverse effects were produced by the highest oral dose tested: 0.44 and 1.88 mg/kg bw/day. By adopting a conservative approach, the lowest value (0.44 mg/kg bw/day) can be considered as a NOAEL. The combined estimated daily *per capita* intake of 2.5 microgram for the 10 candidate substances in subgroup VI corresponds to 0.042 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1 x 10^4 can be calculated. The nine candidate substances, evaluated through the Procedure, in subgroup VI are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup VIII, an adequate 90-day subchronic study is available for one supporting substance, ethyl thioacetate [FL-no: 12.018], which can be considered representative of the eight candidate substances in this subgroup to be evaluated through the Procedure. In the study, no adverse effects were produced by the highest oral dose tested: 6.63 mg/kg bw/day. Therefore, the NOAEL is concluded to be 6.63 mg/kg bw per day for ethyl thioacetate. The combined estimated daily *per capita* intake of 2.4 microgram for the eight candidate substances in subgroup VIII corresponds to 0.04 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.7 x 10^5 can be calculated. The eight candidate substances in subgroup VIII are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup IX, no data are available for the candidate substance ethanethioic acid [FL-no: 12.199]. Therefore, the Panel concluded that additional data are required for the candidate substance in subgroup IX.

The substance in subgroup X is not evaluated through the Procedure, see Section 8.4.

The conclusion from step B4 is that for 46 candidate substances belonging to subgroups I, III, IV, V, VII and VIII, and evaluated through the Procedure, adequate NOAELs exist for structurally related substances providing adequate margins of safety at the estimated levels of intake. Therefore, these candidate substances are not expected to be of safety concern at the levels of exposure estimated by the MSDI approach. For the three candidate substances belonging to subgroup II [FL-no: 12.120, 15.102 and 15.125], the eight candidate substances belonging to subgroup VI [FL-no: FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167] and the candidate substance of subgroup IX [FL-no: 12.199] additional toxicity data are required.

The evaluations of the 66 aliphatic and alicyclic mono-, di-, tri- and polysulphides are summarised in Table 3.2.

4.3. EFSA Considerations

The Panel agrees with the outcome of the application of the Procedure performed by the JECFA for eight out of the 18 simple aliphatic sulphides and thiols, namely [FL-no: 12.179, 12.198, 12.212, 12.238, 12.239, 12.255, 12.257 and 12.291].

In FGE.74 the following evaluation was made for substances 2-(methylthio)ethan-1-ol; 2,3,5-trithiahexane and ethyl-5-(methylthio)valerate [FL-no: 12.179, 12.198 and 12.212]:

The JECFA derives a NOAEL of 1,4 mg/kg bw per day reported in a 13-week study in rats (Cox et al., 1979) fed by gavage with 2-(methylthiomethyl)-3-phenylpropenal [FL-no: 12.087]. The Panel did not agree with the JECFA that 2-(methylthiomethyl)-3-phenylpropenal [FL-no: 12.087] is structurally related to 2-(methylthio)ethan-1-ol [FL-no: 12.179] or ethyl-5-(methylthio)valerate [FL-no: 12.212], and accordingly additional data are required for both substances. The JECFA derives a NOAEL of 0.3 mg/kg bw per day reported in a 13-week study (Mondino, 1981a) in rats fed with 3-methyl-1,2,4-trithiane [FL-no: 15.036]. The Panel does not agree with the JECFA that 2,3,5-trithiahexane [FL-no: 12.198] is structurally related to 3-methyl-1,2,4-trithiane [FL-no: 15.036], and accordingly additional data are required for this substance as well.

In the present revision of FGE.74, FGE.74Rev1, all substances have been distributed to subgroups with respect to sulphur-containing functional groups, according to FGE.08 and FGE.08Rev1. The JECFA evaluated substances 2-(methylthio)ethan-1-ol and ethyl-5-(methylthio)valerate [FL-no: 12.179 and 12.212] have been allocated to subgroup I, *Acyclic sulphides*, and 2,3,5-trithiahexane [FL-no: 12.198] has been allocated to subgroup V, *Acyclic and cyclic disulphides*. Appropriate NOAELs exist for these subgroups, as is demonstrated in FGE.08Rev1. Accordingly the Panel concludes that these substances are not expected to be of safety concern at the estimated levels of intake.

For the remaining 10 substances [FL-no: 12.169, 12.241, 12.280, 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155] the Panel did not agree with the application of the Procedure by the JECFA for the following reasons:

For the two tertiary thiols in the present FGE, both from subgroup III, 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] and 2-mercapto-2-methylpentan-1-ol [FL-no: 12.241], the Panel concluded that in line with the conclusions for 2-methylpropane-2-thiol [FL-no: 12.174], 2-methylbutane-2-thiol [FL-no: 12.172] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057] in FGE.08Rev1, that these two substances should not be evaluated using the Procedure due to concern for genotoxicity. These substances cannot be taken through the Procedure unless the concern for genotoxicity of tertiary thiols has been cleared.

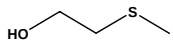
For the eight substances in subgroup VI (acyclic tri- and polysulphides) [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280], 90-day studies were available on [FL-no: 12.009 and 12.023], but the studies were not considered adequate for deriving a NOAEL (Morgareidge & Oser, 1970c; Morgareidge and Oser, 1970d) (see FGE.08Rev1, Section 8.2 (There are no data on stability of test substances and no results reported from histopathological examinations). It has also been concluded that tri- and polysulphides cannot be covered by NOAELs for disulphides, due to the formation of more reactive metabolites than is the case for the disulphides.

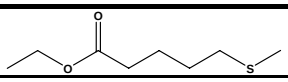
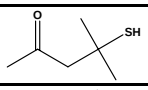
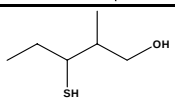
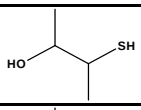
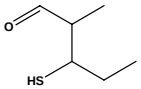
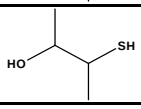
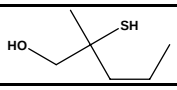
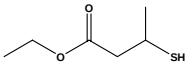
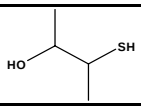
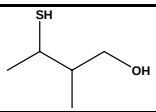
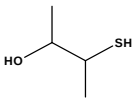
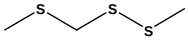
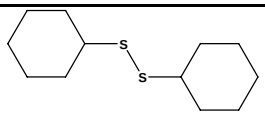
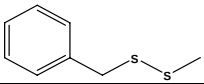
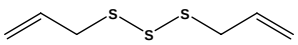
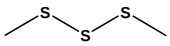
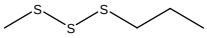
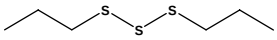
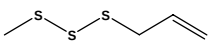
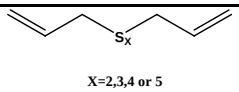
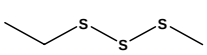
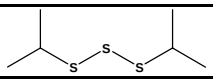
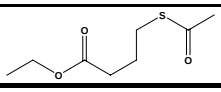
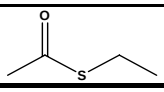
Accordingly, the Panel concluded at step B4 (contrary to JECFA) that further data are required for the tri- and polysulphides [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280].

For two substances [FL-no: 12.045 and 12.155] no European production figures were available and consequently no European exposure estimates could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for these two substances.

An overview of the EFSA considerations is given in Table 4.3 below.

Table 4.3: Overview of Supporting Substances Providing Adequate NOAEL for the Procedure Step B4

FL-no:	Register name	Structural formula	NOAEL provider
I	***Acyclic sulphides***		
12.179	2-(Methylthio)ethan-1-ol		

12.212	Ethyl-5-(methylthio)valerate		
III	***Monothiols***		
12.169	2-Methyl-4-oxopentane-2-thiol		Structural alert for genotoxicity – additional genotoxicity data required
12.238	3-Mercapto-2-methylpentan-1-ol		
12.239	3-Mercapto-2-methylpentanal		
12.241	2-Mercapto-2-methylpentan-1-ol		Structural alert for genotoxicity – additional genotoxicity data required
12.255	Ethyl 3-mercaptobutyrate		
12.291	3-Mercapto-2-methyl-1-butanol		
V	***Acyclic and cyclic disulphides***		
12.198	2,3,5-Trithiahexane		 and 
VI	***Acyclic tri- and polysulphides***		
12.009	Diallyl trisulfide		No adequate NOAEL available for step B4 in the Procedure – additional data required
12.013	Dimethyl trisulfide		No adequate NOAEL available for step B4 in the Procedure – additional data required
12.020	Methyl propyl trisulfide		No adequate NOAEL available for step B4 in the Procedure – additional data required
12.023	Dipropyl trisulfide		No adequate NOAEL available for step B4 in the Procedure – additional data required
12.045	Methyl allyl trisulfide		No European Production volume available preventing the substance to be evaluated using the Procedure
12.074	Diallyl polysulfides	 X=2,3,4 or 5	No adequate NOAEL available for step B4 in the Procedure – additional data required
12.155	Methyl ethyl trisulfide		No European Production volume available preventing the substance to be evaluated using the Procedure
12.280	Diisopropyl trisulphide		No adequate NOAEL available for step B4 in the Procedure – additional data required
VIII	***Thioesters***		
12.257	Ethyl 4-(acetylthio) butyrate		

5. Conclusion

The JECFA has evaluated a group of 12 simple aliphatic sulphides and thiols at the 61st meeting and seven trisulphides in a group of simple aliphatic and aromatic sulphides and thiols at the 53rd meeting.

One of the substances evaluated by the JECFA at its 61st meeting is not in the Register (spiro[2,4-dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3'-(1'-oxa-2'-methyl)-cyclopentane], JECFA-no: 1296). Accordingly this consideration will deal with 18 JECFA evaluated substances.

The Panel concluded that the 18 substances in the JECFA flavouring group of simple aliphatic sulphides and thiols are structurally related to the group of 66 aliphatic and alicyclic mono-, di-, and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in the Flavouring Group Evaluation 08, Revision 1(FGE.08Rev1).

The Panel agrees with the outcome of the application of the Procedure performed by the JECFA for eight of the 18 aliphatic sulphides and thiols [FL-no: 12.179, 12.198, 12.212, 12.238, 12.239, 12.255, 12.257 and 12.291].

The Panel concluded that the two tertiary thiols, 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] and 2-mercapto-2-methylpentan-1-ol [FL-no: 12.241], should not be evaluated through the Procedure, as they are structurally related to three tertiary thiols, 2-methylpropane-2-thiol [FL-no: 12.174], 2-methylbutane-2-thiol [FL-no: 12.172] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057], in FGE.08Rev1 for which the Panel has previously concluded that they could not be evaluated through the Procedure due to concern with respect to genotoxicity *in vitro*.

For the eight tri- and polysulphides [FL-no: FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280] the Panel did not agree with the JECFA that appropiate studies were available for deriving NOAELs, and accordingly the Panel concluded that additional data are required for these eight substances.

For two substances [FL-no: 12.045 and 12.155] the JECFA evaluation is only based on MSDI values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For one substance use levels have been provided by the Industry. The mTAMDI figure calculated for the substances [FL-no: 12.291] is below the threshold of concern for the structural class. For the remaining 17 substances use levels must be provided. These are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 18 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for 10 of the 18 JECFA evaluated substances. For seven substances [FL-no: 12.009, 12.020, 12.045, 12.169, 12.238, 12.239 and 12.291] information on secondary components and/or composition of mixture is requested. For six substances [FL-no: 12.009, 12.020, 12.023, 12.045, 12.074 and 12.155] no solubility in ethanol and/or solubility in water is available. Finally, the European production volumes are not available for [FL-no: 12.045 and 12.155].

Thus, for 10 substances [FL-no: 12.009, 12.020, 12.023, 12.045, 12.074, 12.155, 12.169, 12.238, 12.239 and 12.291] the Panel has reservations (no European production volumes are available, preventing them to be evaluated using the Procedure, and/or information on specifications). For two substances [FL-no: 12.169 and 12.241] the Procedure should not be applied until adequate genotoxicity data become available and for eight substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280] additional toxicity data are required.

For the remaining five of the 18 JECFA evaluated simple aliphatic sulphides and thiols [FL-no: 12.179, 12.198, 12.212, 12.255 and 12.257] the Panel agrees with JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY

Table 1: specifications summary for the 18 JECFA evaluated substances in the present group (JECFA, 2003b; JECFA, 1999c)

Table 1: Specification Summary of the 18 Substances in the JECFA Flavouring Group of Simple Aliphatic Sulphides and Thiols *(JECFA, 2003b; JECFA, 1999c)*

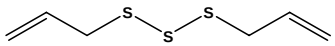
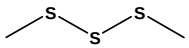
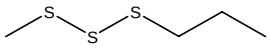
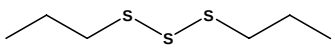
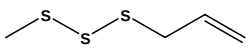
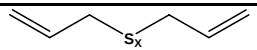
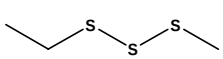
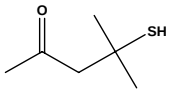
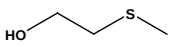
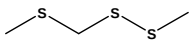
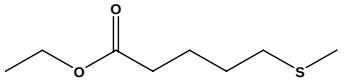
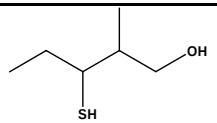
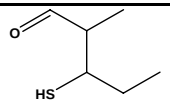
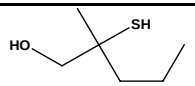
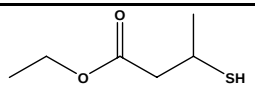
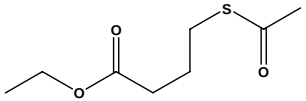
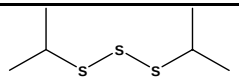
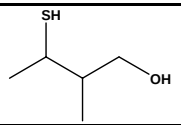
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
12.009 587	Diallyl trisulfide		3265 486 2050-87-5	Liquid $C_6H_{10}S_3$ 178.33	Insoluble	112-120 (21hPa) IR 65 %	1.600-1.620 1.135-1.170	Min. Assay value 65 %, secondary components to be specified.
12.013 582	Dimethyl trisulfide		3275 539 3658-80-8	Liquid $C_2H_6S_3$ 126.26	Very slightly soluble Soluble	165-170 IR 97 %	1.595-1.605 1.195-1.210	
12.020 584	Methyl propyl trisulfide		3308 586 17619-36-2	Liquid $C_4H_{10}S_3$ 154.30		52 (1.6 hPa) IR 45 %	1.558-1.570 1.095-1.101	Min. Assay value 45 %, secondary components to be specified.
12.023 585	Dipropyl trisulfide		3276 726 6028-61-1	Liquid $C_6H_{14}S_3$ 182.36	Almost insoluble	98 (5 hPa) IR 99 %	1.542-1.590 0.952	
12.045 586	Methyl allyl trisulfide		3253 11867 34135-85-8	Liquid $C_4H_8S_3$ 152.29		47 (1 hPa) NMR 80 %	1.593-1.603 0.975-0.985	Min. Assay value 80 %, secondary components to be specified.
12.074 588	Diallyl polysulfides	 X=2,3,4 or 5	3533 11912 72869-75-1	Liquid $C_6H_{10}S_2$ 146.30	Insoluble	68 (20 hPa) IR NMR 95 %	1.643-1.653 1.220 (20°)	
12.155 583	Methyl ethyl trisulfide		3861 31499-71-5	Liquid $C_3H_8S_3$ 140.28	Very slightly soluble	46-47 (5 hPa) NMR 97 %	1.510-1.520 0.955-0.965	
12.169 1293	2-Methyl-4-oxopentane-2-thiol		3997 11500 19872-52-7	Liquid $C_6H_{12}OS$ 132.23	Soluble Soluble	47-49 (20 hPa) IR NMR MS 48 %	1.431-1.437 1.032-1.037	The Register name to be changed to 4-Mercapto-4-methyl-2-pentanone. According to the JECFA: Min. assay value is "48 %" and secondary component "4-methyl-3-penten-2-one; supplied as a 1 % solution in propylene glycol. Composition of mixture to be more specified.

Table 1: Specification Summary of the 18 Substances in the JECFA Flavouring Group of Simple Aliphatic Sulphides and Thiols *(JECFA, 2003b; JECFA, 1999c)*

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
12.179 1297	2-(Methylthio)ethan-1-ol		4004 11545 5271-38-5	Liquid C_3H_8OS 92.16	Insoluble Soluble	169-171 IR NMR MS 98 %	1.490-1.498 1.055-1.065 (20º)	
12.198 1299	2,3,5-Trithiahexane		4021 42474-44-3	Liquid $C_3H_8S_3$ 140.30	Insoluble Soluble	56-58 (10 hPa) MS 95 %	1.436-1.444 1.157-1.163	CASrn in Register to be changed to 42474-44-2.
12.212 1298	Ethyl-5-(methylthio)valerate		3978 233665-98-0	Liquid $C_8H_{16}O_2S$ 176.27	Insoluble Soluble	227 IR NMR MS 96 %	1.460-1.464 0.993-1.003 (20º)	Register name to be changed to Ethyl 5-(methylthio)valerate.
12.238 1291	3-Mercapto-2-methylpentan-1-ol		3996 227456-27-1	Liquid $C_6H_{14}OS$ 134.24	Slightly soluble Soluble	50 (0.7 hPa) IR NMR 99 %	1.480-1.490 0.985-0.995	Composition of stereoisomeric mixture not specified.
12.239 1292	3-Mercapto-2-methylpentanal		3994 227456-28-2	Liquid $C_6H_{12}OS$ 132.23	Insoluble Soluble	98-100 (13 hPa) IR 96 %	1.523-1.529 1.095-1.103	Composition of stereoisomeric mixture not specified.
12.241 1290	2-Mercapto-2-methylpentan-1-ol		3995 258823-39-1	Liquid $C_6H_{14}OS$ 134.24	Slightly soluble Soluble	57-59 (0.8 hPa) IR NMR 99 %	1.476-1.483 0.968-0.974 (20º)	Racemate.
12.255 1294	Ethyl 3-mercaptobutyrate		3977 156472-94-5	Liquid $C_6H_{12}O_2S$ 148.22	Insoluble Soluble	188 IR NMR MS 97 %	1.448-1.453 1.011-1.021 (20º)	Racemate.
12.257 1295	Ethyl 4-(acetylthio)butyrate		3974 104228-51-5	Liquid $C_8H_{14}O_3S$ 190.26	Insoluble Soluble	262 IR NMR MS 96 %	1.468-1.472 1.073-1.083 (20º)	
12.280 1300	Diisopropyl trisulphide		 5943-34-0	Liquid $C_6H_{14}S_3$ 182.40	Insoluble Soluble	107-108(13 hPa) NMR MS 95 %	1.441-1.445 1.134-1.140	
12.291 1289	3-Mercapto-2-methyl-1-butanol		3993 227456-33-9	Liquid $C_5H_{12}OS$ 120.21	Slightly soluble 1 ml in 1 ml	98 (at 2.7 hPa) IR NMR MS 98 %	1.482-1.490 1.002-1.008	Composition of stereoisomeric mixture not specified.

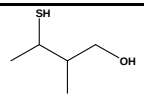
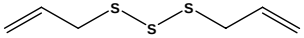
1) Solubility in water, if not otherwise stated.

2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (in vitro / in vivo) for 11 Simple Aliphatic Sulphides and Thiols (JECFA, 2000c; JECFA, 2004b)

Table 2.1: Summary of genotoxicity data of 11 (name of group of substance) evaluted by JECFA

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference	Comments
In vitro								
12.291 1289	3-Mercapto-2-methyl-1-butanol		Reverse mutation	*S. typhimurium* TA1535, TA97, TA98, TA100, TA102	50–5000 µg/ plate	Negative[a]	(Gocke, 1997a)	The racemate (Erythr- and threo-3-Mercapto-2-methyl-1-butanol) was used in the toxicological evaluation.
12.009 587	Diallyl trisulfide		Micronucleus formation	Mouse	59-120 mg/kg bw	Negative	(Marks et al., 1992)	

a With and without metabolic activation from S9

Table 2.2: Genotoxicity Data (in vitro) EFSA / FGE.08Rev1

Substances listed in brackets are JECFA-evaluated substances

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.08Rev1

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
Subgroup I – Acyclic Sulphides						
(Allyl sulphide [12.088])	Ames test	*S. typhimurium* TA100	0.004 – 0.44 µg/ml	Negative (±S9)	(Eder et al., 1982a)	Review. No details on method and results reported. Only TA100 used.
	Sister chromatid exchange	Chinese hamster ovary cells	200 - 600 µg/ml	Positive[1]	(Musk et al., 1997)	Limited quality of study. Insufficiently reported.
	Chromosomal aberrations	Chinese hamster ovary cells	200 - 600 µg/ml	Positive[1]	(Musk et al., 1997)	Limited quality of study. Insufficiently reported.
Di-(1-propenyl)-sulfid (mixture) [12.298]	*Ames test*	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	1 – 100 µg/plate	Negative[1]	(Stien, 2005c)	Un-published GLP study. Study considered valid.
Subgroup II – Cyclic Sulphides						
Tetrahydrothiophene [15.102]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	50 – 5000 µg/plate	Negative (±S9)	(Pennwalt Corporation, 1987a-d)	Validity of this study cannot be fully evaluated (only abstract provided).
	Cytogenetic assay	Human lymphocytes	12.5 – 125 µg/ml	Negative (±S9)	(Pennwalt Corporation, 1987a-d)	Validity of this study cannot be fully evaluated (only abstract provided).
	HPRT assay	Chinese hamster ovary cells	100 – 200 µg/ml	Negative (±S9)	(Pennwalt Corporation, 1987a-d)	Validity of this study cannot be fully evaluated (only abstract provided).
	Sister chromatid exchange	Chinese hamster ovary cells	15.63 – 125 µg/ml	Negative (±S9)	(Pennwalt Corporation, 1987e)	Validity of this study cannot be fully evaluated (only abstract provided).
	Unscheduled DNA synthesis	Human epithelial cells	2.5 – 5120 µg/ml	Negative (±S9)	(Pennwalt Corporation, 1987a-d)	Validity of this study cannot be fully evaluated (only abstract provided).
(1,4-Dithiane [15.066])	Ames test	*S. typhimurium* TA98, TA100	0.8 – 100 µ mol/plate (96.2 - 12024 µg/plate)	Positive (-S9) Negative (+S9)	(Lee et al., 1994a)	Only two strains were tested, otherwise acceptable study.
	Sister chromatid exchange	Chinese hamster ovary cells	2000 µM (240 µg/ml)	Negative (±S9)	(Lee et al., 1994a)	Insufficient quality.
Subgroup III – Monothiols						
2-Methylpropane-2-thiol [12.174]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10000 µg/plate	Negative (±S9)	(Phillips Petroleum Company, 1990a)	Validity of this study cannot be fully evaluated (only abstract provided).
	Forward mutational MLTK assay	L5178Y/tk+/- mouse lymphoma cells	1000 µg/ml	Positive (-S9) Negative (+S9)	(Phillips Petroleum Company, 1990a)	Validity of this study cannot be fully evaluated (only abstract provided).
	Sister chromatid exchange	Chinese hamster ovary cells	1350 µg/ml	Negative (+S9)[2]	(Phillips Petroleum Company, 1990a)	Validity of this study cannot be fully evaluated (only abstract provided).
(Allyl mercaptan [12.004])	Modified Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.005 – 1.5 µl/ml (4.6 – 1400 µg/ml)	Negative (±S9)	(Eder et al., 1980)	Acceptable quality.
(Benzyl mercaptan [12.005])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently documented.
(2-Mercaptopropionic acid [12.039])	Ames test	*S. typhimurium* TA98, TA100,	3.6 mg/plate (3600	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.08Rev1

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
		TA1535, TA1537, TA1538	µg/plate)			documented.
(Benzenethiol [12.080])	Ames test	*S. typhimurium* TA98, TA100	25 – 500 µg/plate	Negative (±S9)	(LaVoie et al., 1979)	Insufficient quality (only two strains were used, and all doses -except the lowest dose - were toxic).
Subgroup IV – Dithiols						
(1,2-Ethanedithiol [12.066])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 doses up to 5000 µg/plate	Negative (±S9)	(Phillips Petroleum Company, 1990b)	Validity cannot be fully evaluated (only abstract provided).
	Sister chromatid exchange	Chinese hamster ovary cells	0.5 - 50 µg/ml	Positive (±S9)	(Pence et al., 1982)	Acceptable quality.
	Forward mutational assay	L5178Y/tk+/- mouse lymphoma cells	150 µg/ml	Positive (-S9)	(Pence et al., 1982)	Positive only at cytotoxic concentrations.
	Forward mutational assay	L5178Y/tk+/- mouse lymphoma cells	1 µg/ml	Negative (+S9)	(Pence et al., 1982)	Insufficiently documented.
Subgroup V – Acyclic and cyclic Disulphides						
(Diallyl disulphide [12.008])	Modified Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.0015 – 0.15 µg/ml	Negative (±S9)	(Eder et al., 1980)	Acceptable quality.
	Sister chromatid exchange	Chinese hamster ovary cells	2 - 25 µg/ml	Weakly positive (±S9)	(Musk et al., 1997)	Limited quality. Insufficiently reported.
	Chromosomal aberrations	Chinese hamster ovary cells	2 - 25 µg/ml	Positive (±S9)	(Musk et al., 1997)	Limited quality. Insufficiently reported.
(Dimethyl disulphide [12.026])	Ames test	*S. typhimurium* TA98, TA100, TA102	0.000011 – 1.1 mmol/plate (1.04 - 104000 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Limited quality (only 3 strains used).
(Phenyl disulphide [12.043])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently documented.
(Benzyl disulphide [12.081])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently documented.
Dibutyl disulphide [12.111]	Forward mutational assay	Mouse lymphoma cells	NR	Negative (-S9)	(Dooley et al., 1987)	Validity cannot be fully evaluated (only abstract provided).
Subgroup VIII – Thioesters						
(Methylthio 2-(acetyloxy)propionate [12.203])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. Coli* WP2uvrA	0.156-5.0 mg/plate (156-5000 µg/plate	Negative (±S9)	(Watanabe & Morimoto, 1989a)	Acceptable quality.
(Methylthio 2-(propionyloxy) propionate [12.227])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. Coli* WP2uvrA	0.156 – 5.0 mg/plate (156 - 5000 µg/plate)	Negative (±S9)	(Watanabe & Morimoto, 1989b)	Acceptable quality.
Subgroup X – Sulfoxides/Sulphones and Sulphonates						
Methyl methane-thiosulfonate [12.159]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538, TA2637	0.6 – 60 µg/plate	Negative (-S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Ames test	*S. typhimurium* TA98, TA100,	2 – 600 µg/plate	Negative (+S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.08Rev1

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
		TA1535, TA1537, TA1538, TA2637				agents[6].
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0.6 – 60 µg/plate	Negative (-S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Ames test	*S.typhimurium* TA98, TA100, TA2637	0.6 – 200 µg/plate	Negative (+S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Ames test	*S.typhimurium* TA98, TA100, TA2637	NR	Negative[3]	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0.6 – 200 µg/plate	Negative[4]	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Yeast assay	*S.cerevisiae* Strain D7	1– 300 µg/ml	Negative (±S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Yeast assay	*S. cerevisiae* Haploid strain N123	1– 100 µg/ml	Negative (±S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
(Methylsulfinyl methane [12.175]) (synonym: dimethylsulfoxid, DMSO)	Ames test	*S. typhimurium* TA97, TA98, TA100	100000 – 300000 µg/plate	Negative (±S9)	(Brams et al., 1987)	Insufficient method (3 strains and 3 concentrations only).
	Ames test	*S.typhimurium* TA97, TA98, TA100, TA1535, TA1537	100 – 10000 µg/plate	Negative (±S9)	(Zeiger et al., 1992)	Acceptable quality.
	Ames test	*S.. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1538, *E. Coli* WP2	0.1 – 0.4 ml/plate (100000 - 400000 µg/plate)	Negative (-S9)	(Hakura et al., 1993)	Good quality study.
	Ames test	*S. typhimurium* TA1537, TA2637, *E. Coli* WP2uvrA	0.1 – 0.4 ml/plate (100000 - 400000 µg/plate)	Positive (-S9) [5]	(Hakura et al., 1993)	Good quality study. Positive at high doses with reduced bacterial survival. Doses routinely used in Ames test were negative.

NR: Not reported.

[1] With and without metabolic activation at clearly cytotoxic concentrations.

[2] A statistically significant increase in the number of SCEs per chromosome was seen at 1350 µg/ml and the 450 µg/ml dose level in the presence of metabolic activation; but no significant increase was seen in the remaining dose levels, and no dose level showed a two fold increase in SCEs; therefore, t-butyl mercaptan is not considered to be mutagenic.

[3] With 100 µl/plate fecalase.

[4] With 100 µl/plate S9 metabolic activation and 100 µl/plate fecalase. Negative results reported after 2 days of incubation. Results for TA98 test strain were positive after 5 days of incubation.

[5] Positive results obtained at doses where lethal toxicity was observed. Negative results obtained at doses routinely used in Ames test.

[6] Thiosulphonates in general, and methyl methane thiosulphonate in particular, are non-specific antimicrobial agents that are active at low concentrations on prokaryotic bacteria, as well as on yeast and other eukaryotic fungi. This was even pointed out by Dorange et al. (1983). Therefore bacterial test systems and yeast assays are not appropriate to evaluate genotoxicity of thiosulphonates.

Table 2.3: Genotoxicity Data (in vivo) EFSA / FGE.08Rev1

Substances listed in brackets are JECFA-evaluated substances

Table 2.3: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
Subgroup I – Acyclic Sulphides							
(Allyl sulphide [12.088])	*In vivo* mouse micronucleus test	Mouse	gavage	0.33 – 0.67 mM/kg (38 – 77 mg/kg)[1]	Negative	(Marks et al., 1992)	Insufficient quality. Mixture of three substances was tested.
Subgroup III – Monothiols							
(2-Mercaptopropionic acid [12.039])	*In vivo* Basc test	Drosophila	dietary route	10 mM (1061 µg/ml)	Negative	(Wild et al., 1983)	Limited quality (insufficiently documented). The article compiles results obtained with 76 substances in 3 test systems.
Subgroup V – Acyclic and cyclic Disulphides							
(Allyl disulphide [12.008])	*In vivo* mouse micronucleus test	Mouse	gavage	0.33 – 0.67 mM/kg (48 – 98 mg/kg) [1]	Negative	(Marks et al., 1992)	Insufficient quality. Mixture of three substances was tested.
Subgroup VI – Acyclic Tri- and Polysulphides							
(Diallyl trisulphide [12.009])	*In vivo* mouse micronucleus test	Mouse	gavage	0.33 – 0.67 mM/kg (59 - 120 mg/kg)[1]	Negative	(Marks et al., 1992)	Insufficient quality. Mixture of three substances was tested.
Subgroup X – Sulphoxides/Sulphones and Sulphonates							
Methyl methane-thiosulfonate [12.159]	*In vivo* genetic mutation	*Nicotiana tabacum* seeds	-	2 - 4 mg/ml (2000 - 4000 µg/ml)	Negative	(Dorange et al., 1983)	Obscure test system[2]. This assay cannot be regarded as standard test.
	In vivo genetic mutation	*Nicotiana tabacum* seeds	-	50 – 400 µg/ml	Negative	(Dorange et al., 1983)	Obscure test system[2]. This assay cannot be regarded as standard test.

[1] Study used a mixture of allyl sulphide, allyl disulphide and ally trisulphide in the respective ratio, 68:20:12.

[2]Heterozygotic seeds were used. After exposure, the seeds were blotted on filter paper and planted in earthenware pots in medium normally used for planting tobacco. The leaves were analysed for alterations indicating genotoxicity.

TABLE 3: SUMMARY OF SAFETY EVALUATIONS

Table 3.1: Summary of Safety Evaluation of Simple Aliphatic Sulphides and Thiols (JECFA, 2004b; JECFA, 2000c)

Table 3.1: Summary of safety evaluation of 18 JECFA-evaluated Simple Sulphides and Thiols (JECFA, 2004b; JECFA, 2000c)

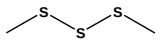
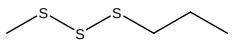
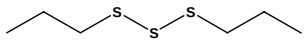
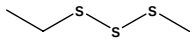
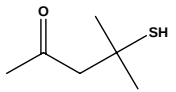
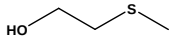
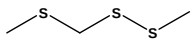
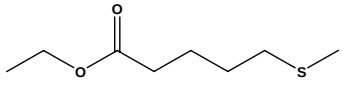
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
12.013 582	Dimethyl trisulfide		1.1 0.02	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Additional toxicity data required	Additional toxicity data required.
12.020 584	Methyl propyl trisulfide		0.21 0.1	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Additional toxicity data required	Additional toxicity data required. Composition of mixture and secondary components to be specified.
12.023 585	Dipropyl trisulfide		7.3 1	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Additional toxicity data required	Additional toxicity data required.
12.155 583	Methyl ethyl trisulfide		ND 1	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Additional toxicity data required	Additional toxicity data required.
12.169 1293	2-Methyl-4-oxopentane-2-thiol		0.0085 0.02	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	2-methyl-4-oxopentane-2-thiol is considered by the EFSA Panel to have genotoxic potential and the Procedure should not be applied until adequate genotoxity data become available	Additional genotoxicity data required. Composition of mixture to be specified.
12.179 1297	2-(Methylthio)ethan-1-ol		0.85 0.9	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
12.198 1299	2,3,5-Trithiahexane		0.026 0.04	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
12.212 1298	Ethyl-5-(methylthio)valerate		1.7 2	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.

Table 3.1: Summary of safety evaluation of 18 JECFA-evaluated Simple Sulphides and Thiols (JECFA, 2004b; JECFA, 2000c)

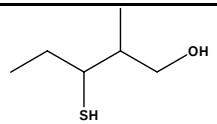
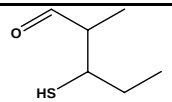
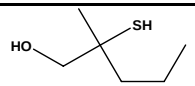
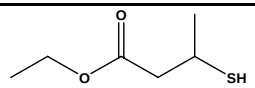
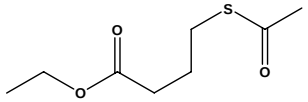
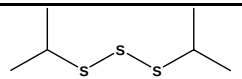
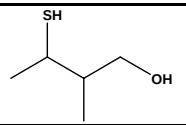
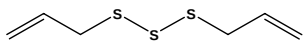
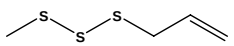
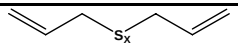
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
12.238 1291	3-Mercapto-2-methylpentan-1-ol		0.85 0.7	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	Composition of mixture to be specified.
12.239 1292	3-Mercapto-2-methylpentanal		2.6 4	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	Composition of mixture to be specified.
12.241 1290	2-Mercapto-2-methylpentan-1-ol		2.6 4	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	2-mercapto-2-methylpentan-1-ol is considered by the EFSA Panel to have genotoxic potential and the Procedure should not be applied until adequate genotoxity data become available	Additional genotoxicity data required.
12.255 1294	Ethyl 3-mercaptobutyrate		3.4 4	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
12.257 1295	Ethyl 4-(acetylthio) butyrate		3.4 4	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
12.280 1300	Diisopropyl trisulphide		0.24 0.007	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Additional toxicity data required	Additional toxicity data required.
12.291 1289	3-Mercapto-2-methyl-1-butanol		0.061 2	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	Composition of mixture to be specified.
12.009 587	Diallyl trisulfide		3.5 0.02	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Additional toxicity data required	Additional toxicity data required. Composition of mixture and secondary components to be specified.
12.045 586	Methyl allyl trisulfide		ND 0.9	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Additional toxicity data required	Additional toxicity data required. Composition of mixture and secondary components to be specified.

Table 3.1: Summary of safety evaluation of 18 JECFA-evaluated Simple Sulphides and Thiols (JECFA, 2004b; JECFA, 2000c)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
12.074 588	Diallyl polysulfides	 X=2,3,4 or 5	1.2 0.02	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Additional toxicity data required	Additional toxicity data required.

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.
ND: not determined.

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.08Rev1)

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

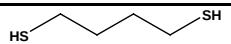
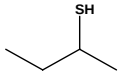
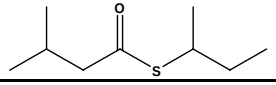
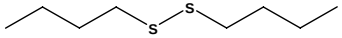
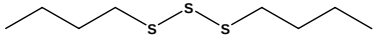
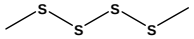
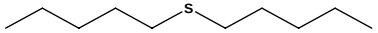
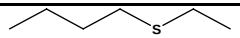
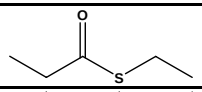
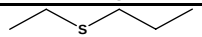
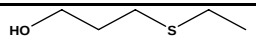
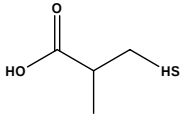
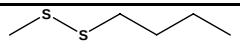
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.103	Butane-1,4-dithiol		0.3	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.104	Butane-2-thiol		0.18	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.106	S-2-Butyl 3-methylbutanethioate		0.8	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.111	Dibutyl disulfide		0.37	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.112	Dibutyl trisulfide		0.12	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.116	Dimethyl tetrasulfide		0.016	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.117	Dipentyl sulfide		0.0037	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.124	Ethyl butyl sulfide		0.037	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.125	Ethyl propanethioate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.127	Ethyl propyl sulfide		0.085	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.129	3-(Ethylthio)propan-1-ol		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.135	3-Mercapto-2-methylpropionic acid		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.151	Methyl butyl disulfide		0.0061	Class I B3: Intake below threshold,	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

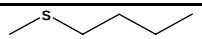
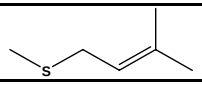
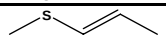
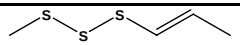
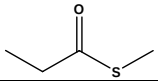
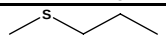
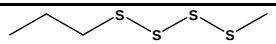
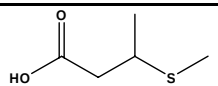
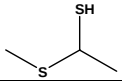
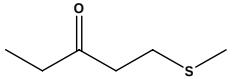
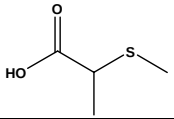
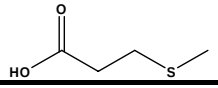
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
				B4: Adequate NOAEL exists			
12.152	Methyl butyl sulfide		0.0024	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.158	Methyl isoprenyl sulfide		0.0012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.163	Methyl prop-1-enyl sulfide		0.0097	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.164	Methyl prop-1-enyl trisulfide		0.0061	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.165 1678	S-Methyl propanethioate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.166	Methyl propyl sulfide		0.0024	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.167	Methyl propyl tetrasulfide		0.0037	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.178	3-(Methylthio)butyric acid		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.180	1-(Methylthio)ethane-1-thiol		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.181	1-(Methylthio)pentan-3-one		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.182	2-(Methylthio)propionic acid		0.011	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.183	3-(Methylthio)propionic acid		0.21	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

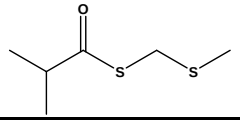
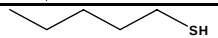
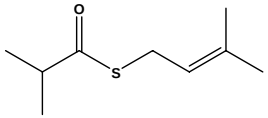
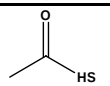
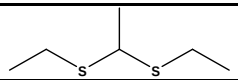
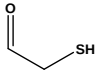
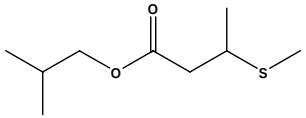
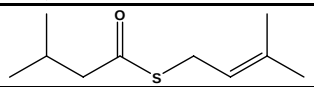
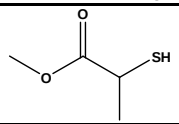
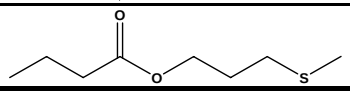
FL-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.189	S-(Methylthiomethyl) 2-methylpropanethioate		0.061	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.191 1662	Pentane-1-thiol		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.196	S-Prenyl thioisobutyrate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.199 1676	Ethanethioic acid		0.0012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.200	1,1-bis(Ethylthio)-ethane		0.0012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.205	Mercaptoacetaldehyde		0.011	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.214 1677	Isobutyl-3-(methylthio)butyrate		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.221	S-Prenyl thioisopentanoate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.266	Methyl-2-mercaptopropionate		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	8)	
12.277	3-(Methylthio)propyl butyrate		6.1	Class I B3: Intake below threshold, B4: No adequate NOAEL	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

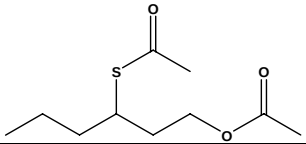
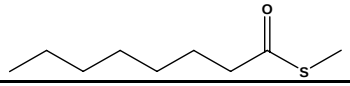
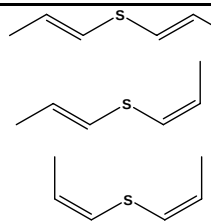
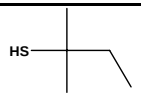
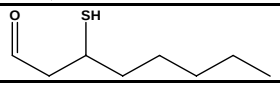
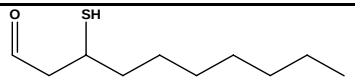
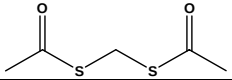
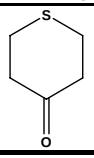
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.278	3-Acetyl-mercaptohexyl acetate		1.2	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.282	(S)-Methyl octanethioate		0.24	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.298	Di-(1-propenyl)-sulfid (mixture)		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.172	2-Methylbutane-2-thiol		0.15	Class I No evaluation			a)
12.174	2-Methylpropane-2-thiol		0.0012	Class I No evaluation			a)
12.268	3-Mercaptooctanal			Class I No evaluation			b)
12.269	3-Mercaptodecanal			Class I No evaluation			b)
12.271	Methanedithiol diacetate			Class I No evaluation			b)
15.125	4-Tetrahydrothiopyranone		0.12	Class II B3: Intake above threshold	Additional data required	8)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

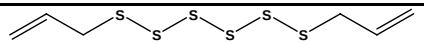
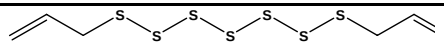
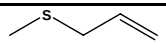
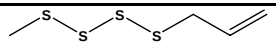
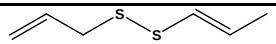
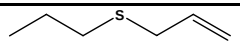
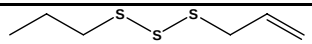
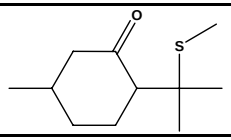
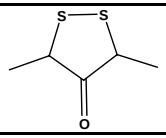
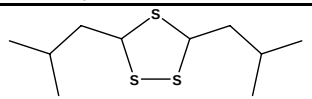
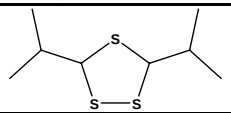
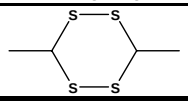
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.093	Diallyl hexasulfide		0.011	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.094	Diallyl heptasulfide		0.011	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.096	Allyl methyl sulfide		0.99	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.097	Allyl methyl tetrasulfide		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.098	Allyl prop-1-enyl disulfide		0.17	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.099	Allyl propyl sulfide		1.6	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.100	Allyl propyl trisulfide		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.177	8-(Methylthio)-p-menthan-3-one		0.37	Class II No evaluation	4)	7)	
12.295	3,5-Dimethyl-1,2-dithiolane-4-one			Class II B3: Intake below threshold, B4: Adequate NOAEL exists		b)	
15.047	3,5-Di-isobutyl-1,2,4-trithiolane		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.048	3,5-Di-isopropyl-1,2,4-trithiolane		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

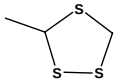
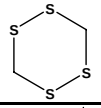
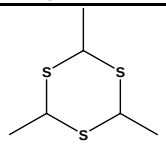
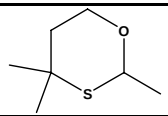
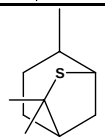
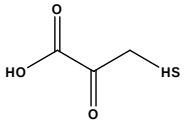
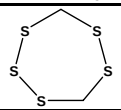
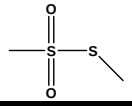
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.083	3-Methyl-1,2,4-trithiolane		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.102	Tetrahydrothiophene		0.024	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.103	1,2,4,5-Tetrathiane		0.073	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.110	2,4,6-Trimethyl-1,3,5-trithiane		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.111	1,2,4-Trithiolane		2.4	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
16.057	2,4,4-Trimethyl-1,3-oxathiane		0.0012	Class II No evaluation			a)
12.120 1685	2,8-Epithio-p-menthane		3.7	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.136	3-Mercapto-2-oxopropionic acid		0.24	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.081	Lenthionine		0.012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.159	Methyl methanethiosulfonate		0.061	Class III No evaluation			a)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day
2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach)
7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.
a) Evaluation deferred pending *in vivo* genotoxicity data.
b) Evaluation deferred pending tonnage data.

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ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practise
ID	Identity
Ip	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
WHO	World Health Organisation